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Phylogeny and morphology of *Ellismarsporium parvum* and the new combination *E. varium*

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Abstract—LSU DNA sequence analyses place *Ellismarsporium parvum* in *Pleosporales*. The morphology of the species in pure culture is described and illustrated. *Helminthosporium varium* is recombined as *E. varium*, based on its morphology.

Key words—*Ascomycota*, asexual fungi, hyphomycetes, phylogeny, taxonomy

Introduction

Ellismarsporium R.F. Castañeda & X.G. Zhang, typified with *E. hypselodelphyos* (M.B. Ellis) R.F. Castañeda & X.G. Zhang is characterized by macronematous and mononematous unbranched conidiophores that

bear integrated and intercalary polytretic conidiogenous cells (Castañeda–Ruiz & al. 2017). The phragmosporic distoseptate conidia are produced in acropetal unbranched or branched chains. Production of conidial chains is the main diagnostic character distinguishing *Ellismarsporium* from *Helminthosporium* Link typified by *H. velutinum* Link. Phylogenetic LSU sequence analysis of strains of *E. parvum* and *Helminthosporium* species has provided additional information on the differences between these genera.

Materials & methods

Pure cultures of *Ellismarsporium parvum* from INIFAT WDCM 853 were transferred to sterilized Petri dishes containing V8 agar (125 ml V8 juice, 18 g agar, 1000 ml distilled water, pH 6.3). Plates were incubated at 25°C in a 12 h dark/light cycle using a box equipped with near UV (black light) VICA FLB-20W T10 lamps. Colony colors were coded according to Rayner (1970). Mounts were prepared in PVL (polyvinyl alcohol, lactic acid) and structures were measured at ×1000 under a Nikon Eclipse Ni-U microscope with bright field optics and photographed using DIC optics with a Nikon DS-Fi2 camera at Universidade Federal de Pernambuco, Recife, Brazil.

Table 1. LSU sequences of *Ellismarsporium parvum* and allied species used in the phylogenetic analysis. New sequences are indicated in **bold.**

Species	Culture	GenBank	Reference
Anteaglonium abbreviatum	GKM219N	GQ221881	Mugambi & Huhndorf 2009
A. parvulum	SMH5210	GQ221907	Mugambi & Huhndorf 2009
Clohesyomyces aquaticus	MFLUCC11-0092	JX276950	Zhang & al. 2012
Corynespora cassiicola	C13-1	KF590123	Palomares-Rius & al. 2014
	SK01	LC177365	Chairin & al. 2017
C. pseudocassiicola	CPC 31708	MH327830	Crous & al. 2018
C. smithii	L133 18S	KY984299	Voglmayr & Jaklitsch 2017
C. torulosa	CPC 15989	KF777207	Crous & al. 2013
Ellismarsporium parvum	**INIFAT-2457**	**MN032445**	**This study**
	INIFAT-2483	**MN032446**	**This study**
	CBS 190.95	MH874149	Vu & al. 2019
Flavomyces fulophazii	CBS 135761	KP184040	Knapp & al. 2015
Fuscostagonospora cytisi	MFLUCC 16-0622	KY770978	Crous & al. 2018
F. sasae	KT 1467	AB807548	Tanaka & al. 2015
Helminthosporium asterinum	CBS 203.35	MH867156	Vu & al. 2019
H. caespitosum	L151	KY984306	Voglmayr & Jaklitsch 2017
H. dalbergiae	H 4628	AB807521	Tanaka & al. 2015
H. genistae	L144	KY984312	Voglmayr & Jaklitsch 2017
H. leucadendri	CBS 135133	KF251654	Quaedvlieg & al. 2013
H. massarinum	KT 838	AB807523	Tanaka & al. 2015

H. magnisporum	H 4627	AB807522	Tanaka & al. 2015
H. microsorum	L96	KY984329	Voglmayr & Jaklitsch 2017
H. oligosporum	L93	KY984333	Voglmayr & Jaklitsch 2017
H. quercinum	L107	KY984336	Voglmayr & Jaklitsch 2017
Hongkongmyces pedis	HKU 35	NG 056287	Tsang & al. 2014
H. thailandica	MFLUCC 16-0406	KY771321	Crous & al. 2018
Keissleriella cladophila	UTHSC:DI16-326	LN907469	Valenzuela-Lopez & al. 2017
K. culmifida	KT 2308	AB807591	Tanaka & al. 2015
	KT 2642	AB807592	Tanaka & al. 2015
K. gloeospora	KT 829	AB807589	Tanaka & al. 2015
Kirschsteiniothelia aethiops	CBS 111.56	MH869065	Vu & al. 2019
	CBS 118.59	MH877780	Vu & al. 2019
	CBS 111.61	MH869545	Vu & al. 2019
Lentithecium carbonneanum	CBS 144076	MH062991	Crous & al. 2018
L. clioninum	KT 1149A	AB807540	Tanaka & al. 2015
L. lineare	IFRD 2008	FJ795435	Zhang & al. 2009
L. pseudoclioninum	KT 1113	AB807545	Tanaka & al. 2015
L. unicellulare	MD6004	KX505376	Crous & al. 2018
L. voraginesporum	H-22560	KX499520	Crous & al. 2018
Lindgomyces ingoldianus	ATCC 200398	AB521736	Hirayama & al. 2010
L. rotundatus	MAFF 239473	KF314114	Tsang & al. 2014
Magnicamarosporium iriomotense	KT 2822	AB807509	Tanaka & al. 2015
Monocillium mucidum	CBS 980.70	MG826851	Crous & al. 2018
Noosia banksiae	CPC:17282	JF951167	Crous & al. 2011
Periconia cookei	MFLUCC 17-1399	MG333493	Hyde & al. 2018
P. cyperacearum	CPC 32138	MH327851	Crous & al. 2018
P. digitata	CBS 510.77	AB807561	Tanaka & al. 2015
P. homothallica	KT 916	AB807565	Tanaka & al. 2015
P. igniaria	CBS 845.96	AB807567	Tanaka & al. 2015
P. macrospinosa	CBS 135663	KP184038	Knapp & al. 2015
P. pseudodigitata	KT 1395	AB807564	Tanaka & al. 2015
P. thailandica	MFLUCC 17-0065	KY753888	Liu & al. 2017
Pleurophoma acaciae	CPC 29188	KY173524	Crous & al. 2013
Preussia fleischhakii	CBS 565.63	GQ203721	Kruys & Wedin 2009
P. terricola	CBS 527.84	GQ203724	Kruys & Wedin 2009
P. typharum	CBS 107.69	GQ203726	Kruys & Wedin 2009
Pseudodictyosporium thailandica	MFLUCC 16-0029	KX259522	Wang & al. 2016
Spadicoides bina	CBS 137794	KY931824	Réblová & al. 2018
S. fuscolutea	CBS 141263	KY931826	Réblová & al. 2018
S. hyalostoma	CBS 139771	KY931830	Réblová & al. 2018
Sporidesmium tengii	HKUCC 10837	DQ408559	Shenoy & al. 2006
Sporormia fimetaria	UPS: Lundqvist 2302-c	GQ203728	Kruys & Wedin 2009
Sporormiella megalospora	UPS:Kruys 305	GQ203743	Kruys & Wedin 2009

DNA extraction, amplification, alignment, and phylogenetic analysis

The INIFAT-2483 and INIFAT-2457 isolates were cultured on V8 for 10 days at 25°C under near UV in a 12 h dark/light cycle. DNA extraction followed a modified protocol from Cenis (1992). The primer pair LROR/LR5 was used to amplify the D1/D3 domain of the LSU nrDNA (White & al. 1990). PCR products were purified and sequenced at Macrogen Inc., South Korea. Consensus sequences assembled and edited using Geneious (ver. 10.1.2) were compared with those of the National Center for Biotechnology Information (NCBI) using BLAST.

Alignments for each data set were made in MEGA 6.0 (Tamura & al. 2013) using the ClustalW algorithm (Thompson & al. 1994) and refined with MUSCLE (Edgar 2004). The alignment included our strain sequences and others representing different genera in *Pleosporales* available from NCBI (Table 1). A LSU sequence phylogeny was generated using Maximum Likelihood (ML) with the best nucleotide substitution model found in MEGA 6.0 (Tamura & al. 2013). The best model used was Tamura-Nei with Gamma distribution. Bootstrap analysis of 1000 replicates assessed the reliability of the reconstructed phylogenies. ML bootstrap values ≥70% were considered significant.

Results

The BLAST query revealed that LSU sequences of *E. parvum* INIFAT-2483 and INIFAT-2457 showed a 99.3% similarity with *E. parvum* [≡ *Helminthosporium parvum*, ex-type] CBS 190.95 and 92.1% with *Helminthosporium asterinum* Cooke CBS 203.35. However, they showed sequence identity of <86% with LSU sequences of other *Helminthosporium* species.

We carried out a phylogenetic analysis of the LSU locus to assess the relationships of *Ellismarsporium* with members of the *Pleosporales*. The final analysis encompassed 62 sequences and comprised 552 bp. The ML tree nested all *E. parvum* isolates (INIFAT-2483, INIFAT-2457, CBS 190.95) in a well-supported subclade (bootstrap value = 99%), with *H. asterinum* CBS 203.35 and *Kirschsteiniothelia aethiops* (Sacc.) D. Hawksw. as the closest sister taxa (Fig. 1).

Both morphological and phylogenetic analyses of *E. parvum* support *Ellismarsporium* within *Pleosporales*.

Taxonomy

Ellismarsporium parvum R.F. Castañeda & W.B. Kendr., Mycotaxon 132(4): 763 (2017) Figs 2, 3

≡ *Helminthosporium parvum* R.F. Castañeda & W.B. Kendr., Univ. Waterloo Biol. Ser. 35: 57 (1991), nom. illeg., non Grove (1886)

Colonies on V8 agar at 25°C attaining 24 mm diam after 10 days, felted, sparsely minutely lanate, greenish olivaceous. Conidiophores

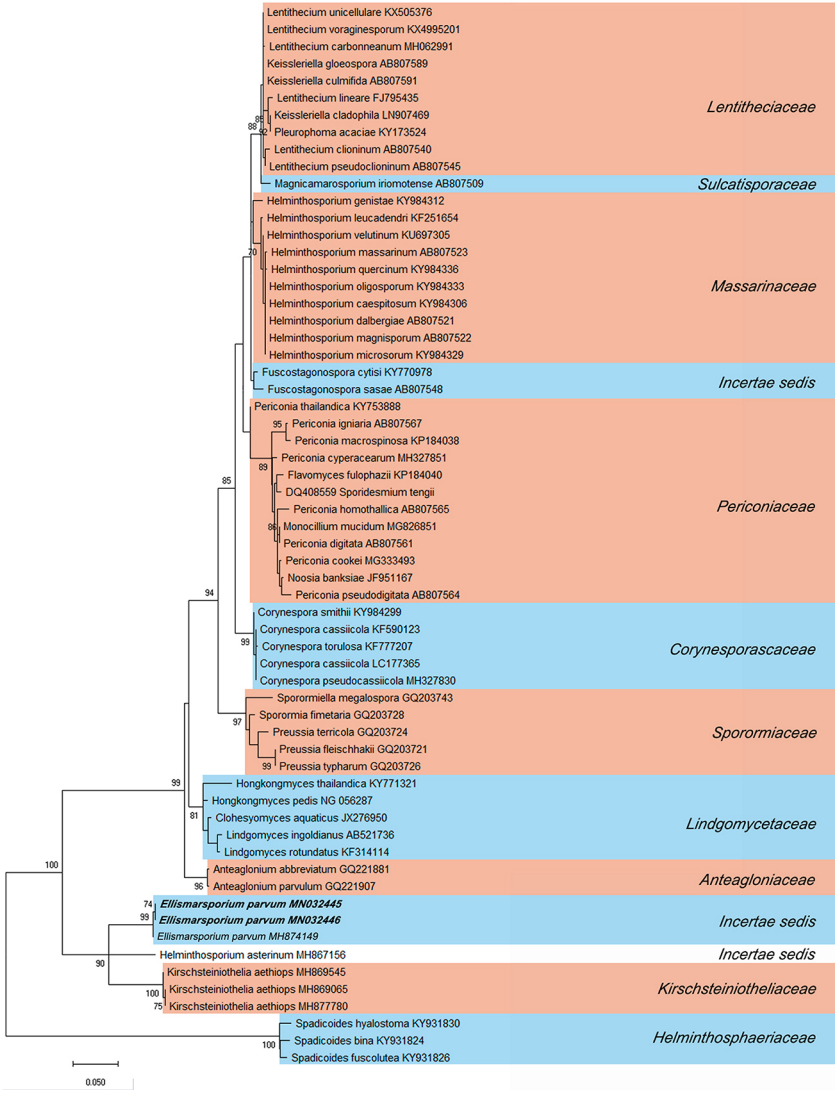

Fig. 1. Maximum Likelihood (ML) tree inferred from LSU sequences of *Ellismarsporium parvum* and related genera, based on 62 sequences. The bootstrap test was conducted with 1000 replicates; bootstrap values ≥70% are indicated at the nodes. The scale bar indicates the number of expected changes per site.

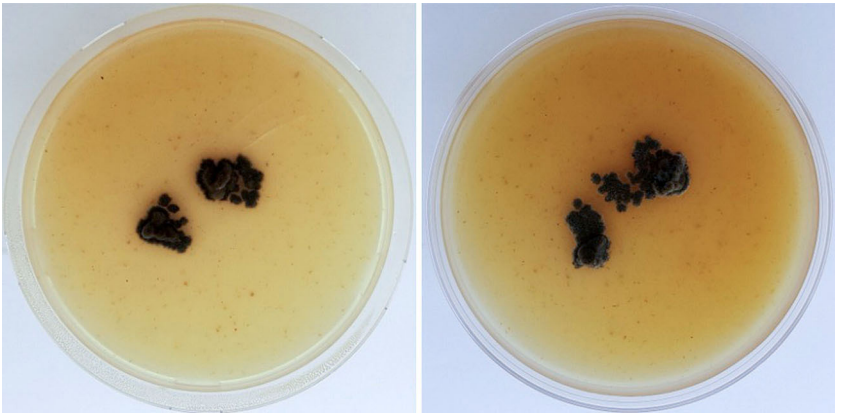

Fig. 2. *Ellismarsporium parvum* (holotype, INIFAT C90/101). Colonies on V8 agar.

macronematous, mononematous, erect, straight or flexuous, unbranched, cylindrical, sometimes slightly clavate or inflated toward the apex, 4–10-septate, brown, smooth, ≤220 µm long. Conidiogenous cells polytretic, intercalary and terminal, integrated, cylindrical (terminal cells somewhat clavate to inflated or slightly curved), brown, 10–40 × 3–8 µm. Conidia acropleurogenous, cylindrical-ellipsoidal, cylindrical to oblong (rarely obovoid), 1–2-distoseptate, smooth, brown to smoke-olivaceous-brown, 14–23 × 6–10 µm, in short branched acropetal chains.

Specimens examined: **CUBA, Pinar del Rio Province, San Juan y Martínez**, San Simón de las Cuchillas, 22°16′N 83°50′W, on bark of *Quercus oleoides* var. *sagrana* (Nutt.) C.H. Mull. [= *Q. cubana* A. Rich.], 24 March 1990, R.F. Castañeda Ruiz (holotype, INIFAT C90/101; INIFAT-2457, GenBank MN032445 [URM 94323 & CCMCIBE-H500]; INIFAT-2483, GenBank MN032446 [URM 94322 & CCMCIBE-H501]).

Helminthosporium varium, published by Alves-Barbosa & al. (2017), is distinguished by distoseptate, long obclavate, subcylindrical, oblong, or navicular, 1–4-septate, irregularly verrucose or verruculose, gray brown to brown conidia produced in branched and unbranched acropetal chains. As the species clearly falls within the *Ellismarsporium* generic concept, we propose a new combination:

Ellismarsporium varium (Alves-Barb., Malosso & R.F. Castañeda) K. Zhang, Alves-Barb., Malosso & R.F. Castañeda, **comb. nov.**
MB 832010
≡ *Helminthosporium varium* Alves-Barb., Malosso & R.F. Castañeda, Nova Hedwigia 105: 67 (2017)

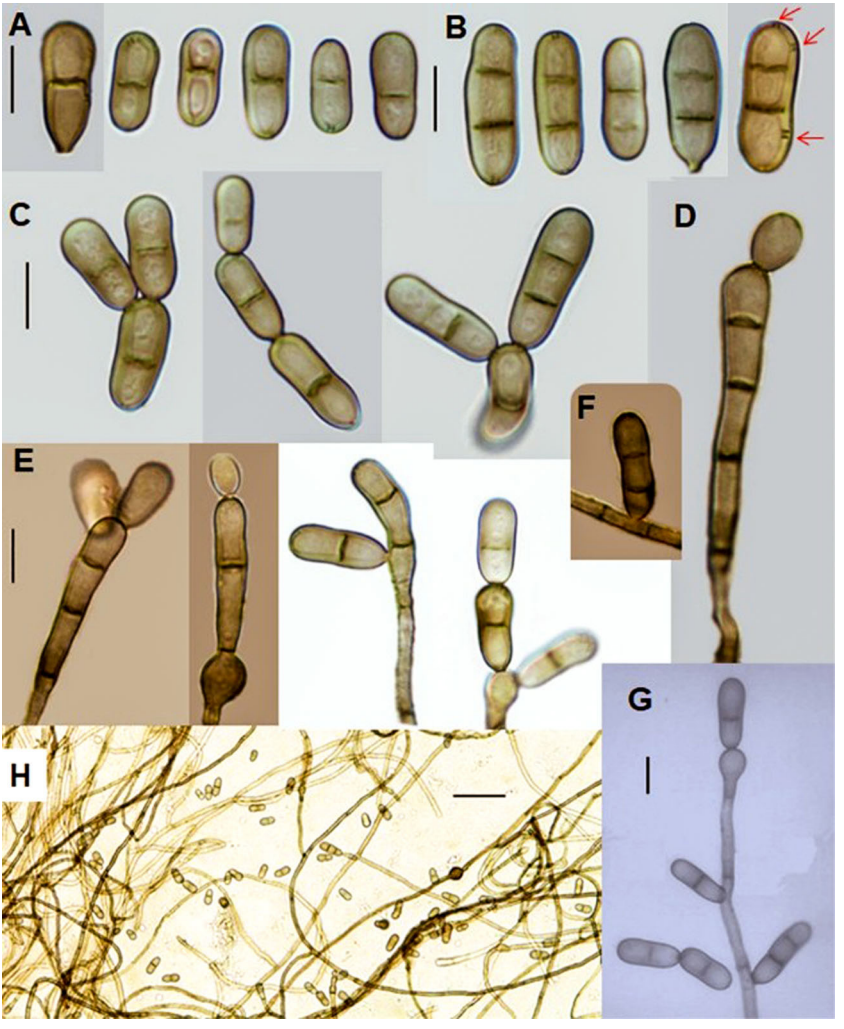

Fig. 3. *Ellismarsporium parvum* (from culture ex holotype INIFAT C90/101). A–C. Conidia; D–G. Conidiogenous cells and conidia; H. Hyphae, conidiophores, conidiogenous cells, and conidia. Scale bars: A–G = 10 µm; H = 50 µm.

Discussion

The phylogenetic placement of *Ellismarsporium* was previously unknown, despite several studies on the phylogenetic relationships of *Pleosporales*

(Hashimoto & al. 2017, Li & al. 2017). Our phylogenetic analysis places *Ellismarsporium* in *Pleosporales*. However, its placement at the family level remains unresolved. Our results show *Ellismarsporium* as sister to the clades of *K. aethiops* (*Kirschsteiniotheliaceae*) and *H. asterinum* CBS 203.35 (Fig. 1). As *Ellismarsporium* was proposed in 2017, it is understandable that the genus was not included in recent phylogenetic studies on *Kirschsteiniotheliaceae* (Su & al. 2016, Mehrabi & al. 2017, Bao & al. 2018). To determine the placement of *Ellismarsporium* at family level, a study using multiple loci is necessary in the future. Additionally, our results place *H. asterinum* CBS 203.35 distant from the *Helminthosporium* clade (Fig. 1), indicating that species also requires further study.

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